



Research Article

Efficacy of Edible Chitosan Formulation in Quality Maintenance and Shelf Life of Guava (*Psidium guajava* L.) Fruit During Cold Storage

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Abstract | Guava is crop of tropical and subtropical regions, but the consumption time is three or four days, so it cannot be sold in a distant market. This trial was carried out to investigate the role of chitosan coating on partially mature guava to retain good quality and prolong shelf life in cold (11°C). Different chitosan concentrations *i.e.* 20% 30% and 40% were coated and stored at 11°C temperature. These fruits were compared with control (non-treated) fruit after 15 days. It was observed that the fruits coated with 40% chitosan showed minimum weight losses of 18.66% at cold storage (11°C) respectively as compared to control where weight losses were 34.50% at cold storage respectively. Treated fruits were firmer (41.67 N) after 15 days storage period as compared to control. Fruit chilling injury percentage was also minimum at cold storage (12.67%) in fruits coated with 40%. All the treatments exhibited potential to maintain the post-harvest life of fruits as compared to untreated fruits. These were found effective in quality attributes such as TSS, titratable acidity, total sugars, total phenolic compounds as well as antioxidants.

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Introduction

Guava (*Psidium guajava* L.) has distinct status in Pakistan and ranked forth on the base of area 63,739 ha and 488,017 tons production. Soil and climate of Punjab and Sindh most suitable for guava production. Punjab is assuming crucial part in production of guava with 373007 tones from an area of 52,462 ha (Anonymous, 2015).

Guava is high nutritional because in light of the fact that its ascorbic acid (50-300 mg 100g⁻¹) is three to six times more as compare to citrus (Mercadante *et al.*, 1999). Its pulp has great action as cancer prevention agent (Musa *et al.*, 2010). But, harvested guava can show respiration ratio high and fast maturing which leads to delicate throughout storing period.

Guava fruit will certainly damage if preserved for three to five days in an ambient temperature (Kumar *et al.*, 2012). A few methods are there to retain the guava fruit's value are; storage at low temperature, controlled atmosphere, the utilization of coating on fruit surface, and chemical material. The temperature throughout the storage significantly influences on fruit's physiology. Respiration rate works fast due to high temperature that will correspondingly accelerate the fruit loss. Low temperature storage expects to reduce respiration, minimize the attack of microorganism and transpiration. Cold temperature storage is a viable and proficient effort since it will reduce respiration through limiting enzyme reaction.

Chitosan is get from prawn shell remaining which has truly great intensity as fruit surface covering

agent. Earlier literature demonstrate that chitosan has qualities to shape film, antimicrobial, and decomposable (Bourtoom *et al.*, 2008).

Furthermore, chitosan has high potential as an edible and decomposable coating in food stuff packaging (Arvanitoyannis *et al.*, 1998), has tremendous biocompatibility, non-toxicity (Jayakumar *et al.*, 2005; Prabakaran *et al.*, 2005) and also has layer making and blockade qualities (Arvanitoyannis *et al.*, 1998), which makes it possible for eatable layer. Past studies described that chitosan covering enhance the storage of various delicate fruits, for example longan (Kaur *et al.*, 2017), peach (Li *et al.*, 2001) and grapes (Romanazzi *et al.*, 2002). Moreover, various examinations have evidently demonstrated that chitosan can be utilized as a successful additive to enhance the quality and time span of usability of different fruits (Meng *et al.*, 2008). So, main purpose of this study was to evaluate prospective impact of chitosan treatments on shelf life of guava cultivar 'Gola' and quality aspects in cold storage.

Materials and Methods

Fruit material

Guava Cv. 'Gola' were picked from an orchard of Horticultural Research institute and Post Harvest Research center AARI, Faisalabad during year 2018-2019. Uniform fruit were separated and selected for research.

Preparation of chitosan formulation

Chitosan solutions were made with the method of Jiang *et al.* (2001). To make the 500 ml of 0, 20%, 30% and 40% (w/v) chitosan solution, exact weight of 0, 100, 150 and 200 g of chitosan was added in 500 ml of glacial acetic acid correspondingly, before 400 ml of double distilled water was added to dissolve more chitosan. The pH maintained at 6.0 with 1M NaOH and the solution prepared up to 500 ml.

Treatments

Fruit dipped into different solutions of 0 (control), 20%, 30% and 40% chitosan for 1 minute, respectively. Fruits kept for 30 minutes at ambient temperature and put into plastic bags and all bags having five fruits with 10 bags each group, and then kept for cold storage (11°C) with 90-95% relative humidity.

All evaluations were showed with three replications.

After storage fruits were continuously examined visually on daily basis to observe the changes in appearance. After 4 weeks of storage the fruits were analyzed on following parameters.

Measurement of fruit color, Firmness, weight loss and chilling injury

Fruit color was carefully examined of each fruit in all replications using horticultural chart and scoring was allocated to each fruit the average was computed. Firmness was measured by Penetrometer and then average was calculated. Weight loss was calculated by formula: $(A-B)/A \times 100$, where A is the before storage fruit weight and B is the after cold storage fruit weight.

Chilling injury and fruit rotting were noted according to treatments. Daily basis fruit rotting occurrence was noted and chilling injury incidence was recorded after 7 days. Rotted fruits were discarded from store and percentage was considered on 4 weeks after storage.

Measurement of Total Soluble Solids, Titratable Acidity, Ascorbic Acid, Total Sugars, Reducing sugar, Non Reducing Sugars, Total Phenolic compounds and Antioxidants

TSS of juice was estimated via digital refractometer (Atago-Palette PR101, Tokyo, Japan). To conclude titratable acidity, fruit juice titrated against 0.1M NaOH solution and results were provided in percentage. Vitamin C of juice was calculated by method of Ruck (1969) and unit mentioned as mg 100 ml⁻¹.

1. Total sugars, reducing and non-reducing sugars were determined by Lane and Eynon method as defined by Ranganna (2008).
2. Total phenolic contents were determined through Folin-Ciocalteu reagent and absorbance read at 765nm using method Singleton *et al.* (1999).
3. Total antioxidants were determined by 50µl extract was added into 5ml 0.004% (4mg/100 ml) of methanol solution of DPPH. Afterward for 30 mins kept in incubation at room temperature and afterward absorbance was estimated at 517nm.

Research was designed according to completely randomized design along with three replications. Each treatment contained ten fruits. Data was evaluated statistically by Statistix software. Analysis of variance technique was used to check total significance of data whereas to relate the difference among treatment

means applied least significance difference (LSD) test.

Results and Discussion

Fruit color

Regarding fruits stored in cold storage (11°C) after 15 days most color retained with 40% (T₃) having an average score color of 1.38. Control (T₀) and 20% chitosan (T₁) showed most changes in color tending towards yellow by average score of 2.47 and 2.51 after 15 days respectively.

Coatings are utilized as a semi permeable boundary to delay colour loss and browning by enzymatic reaction or drying out which are a piece of the cutting procedure reaction (Prabaharan *et al.*, 2006). Though, no significant difference was found about color change amongst control and treated strawberry fruits applying various concentrations of chitosan (Rocha *et al.*, 2007). In past investigation, it was discovered that papaya coated with chitosan showed maximum color in the storage duration (Gonzalez-Aguilar *et al.*, 2009) (Figure 1).

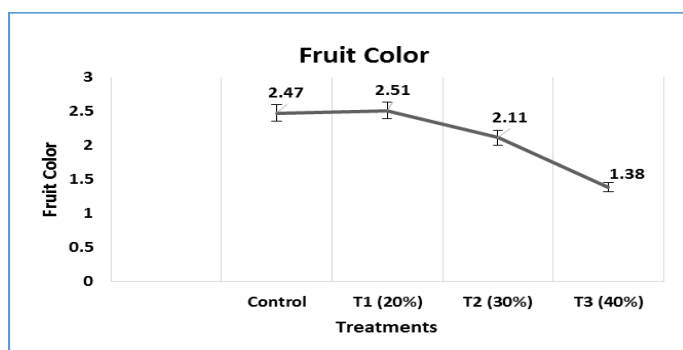


Figure 1: Effect of Chitosan coatings on fruit color of guava.

Fruit weight loss (%)

Chitosan reduced the weight loss percentage which was recorded significantly low 18.66 % in 40% and significantly high (34.00%) in control and treated fruits (Figure 2).

Chitosan coating evidently a obstacle for surface evaporation; consequently, a reduced weight reduction in coated fruit.

These results are in line with earlier worker Krishna and Rao (2014) where the loss of weight of the guava fruit can be lessened by covering it with chitosan. In addition to guava fruit, chitosan coating has been compelling in adjusting reduction of weight of differ-

ent crops, including longan fruit. Jiang *et al.* (2001) and strawberry fruit (Gonzalez-Aguilar *et al.*, 2009). The loss in weight of fresh horticultural crops is mostly due to transpirational water loss (Zhu *et al.*, 2008) (Figure 2).

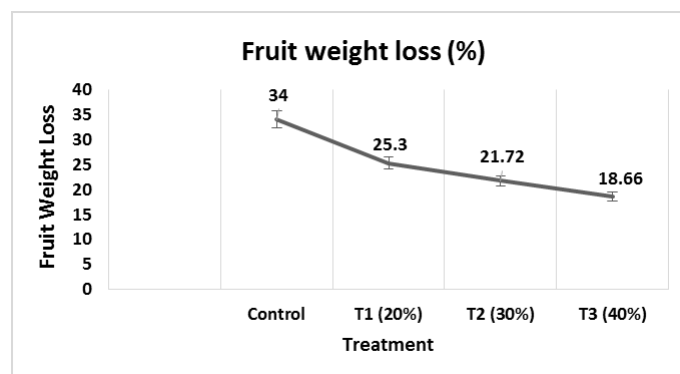


Figure 2: Effect of chitosan coating on weight loss (%) of guava.

Fruit firmness

The fruit firmness is a vital morphological factor to examine the ripening progress. The fruits treated with chitosan 40% recorded maximum firmer fruits (6.67 N) after 15 days, while in control it was minimum (3.88 N). Cold storage condition revealed slow rate of loss of fruit firmness. The present findings are in accordance with work of Krishna and Rao (2014) in guava fruits. Results of firmness retaining results are corresponding to the earlier worker (Ali *et al.*, 2011) who described that 2.0% chitosan coating on papaya retained the firmness than other treatments at low temperature storage (11°C).

In our experiment, with increasing the chitosan concentrations fruit softening was lessened and subsequently, treated fruit with control and 20% chitosan vanished their textural integrity quicker as compare to fruit treated with 30 and 40 mg chitosan.

Fruit softening occurs due to cell structure disintegration, the cell wall structure and ingredients (Scanavaca *et al.*, 2007). Firmness of guava can be conserved due to fruit treated with chitosan that have high antifungal action and layer of the lenticels and cuticle, along these decreasing contamination, respiration and further maturing developments during storage, as indicated by past research on sweet cherry and papaya treated with chitosan and aloe vera gel (Scanavaca *et al.*, 2007; Seymour, *et al.*, 1993) (Figure 3).

Chilling injury percentage was recorded subsequently 15 days of cold storage (11°C). Figure 4 revealed

that fruits coated with 40% and 30% chitosan stored at cold storage (11°C) had less chilling injuries. Both had 12.67% and 13.33% decay after 15 days of storage respectively. Maximum chilling injuries were observed in control treatment (T0) which showed 30% of chilling injuries. Our findings were supported by earlier workers on papaya (Gonzalez-Aguilar *et al.*, 2009) and litchi (Dong *et al.*, 2004).

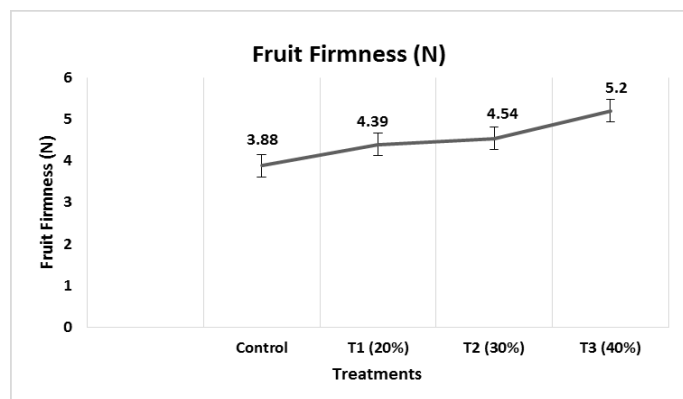


Figure 3: Effect of chitosan coating on fruit firmness of guava Chilling Injury.

In this study, browning of control and 20% chitosan coated samples limited restrict the time span of usability in term of visual quality. These browning can results because of dehydration or microbial contamination or activity of PPO enzymes that are unregulated by the cutting procedure and oxidize phenols into quinines within the sight of sub-atomic oxygen. Sliced mango quality and prolonged shelf life or peeled litchi fruit were also described to be due to the chitosan coating forming film and protect the fruit surface (Dong *et al.*, 2004).

Correspondingly, in this work, the increasing and adequate amount of 40% chitosan on guava improved its quality and prevented decay development. The current study showed that the resistance of the surface to contamination can be achieved by the coating to delay contamination (Figure 4).

Total soluble solids

Total soluble solids was also influenced significantly with days of storage conditions and treatments. As for as fruits coated with chitosan and stored at cold temperature after 15 days are concern total soluble solid of fruits coated with T2 chitosan (30%) had maximum total soluble solids of 8.33 °Brix followed by T3 (40%) which showed 8.00 °Brix (Figure 4 and 2). T1(20%) revealed non-significant results with control (T0) by showing 5.80 °Brix after 15 days of storage.

Consequences of this study are very close with results of earlier workers (Yueming *et al.*, 2005). During fruit ripening TSS increased because activity of enzymes improved and these enzymes do hydrolysis of starch to sugars (Hernández-Munoz *et al.*, 2008) (Figure 5).

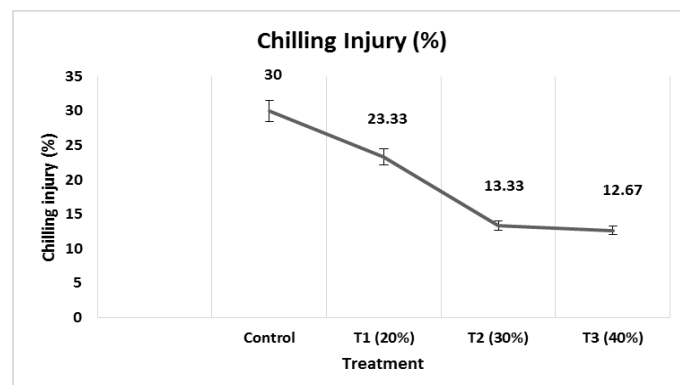


Figure 4: Effect of chitosan coating on fruit chilling injury of guava.

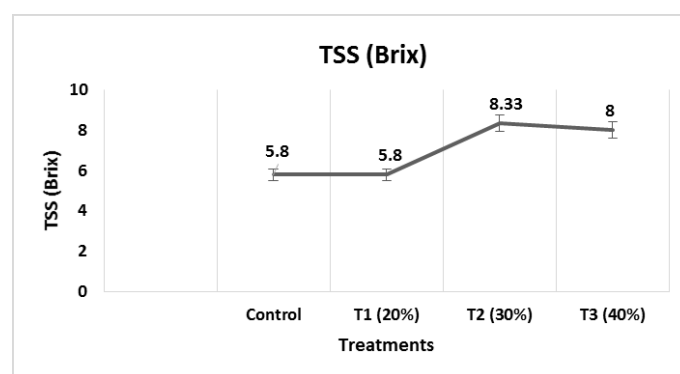


Figure 5: Effect of chitosan coatings on total soluble solids of guava. Titratable acidity.

Titrateable acidity was gradually reduced all treatments throughout the storage period (Figure 6). Maximum total titrateable acidity was observed in T3 (40%) which showed 0.30% total titrateable acidity while T1 showed intermediate value 0.28%. Least aggregate titrateable acidity was seen by applying T0 (control) which gave 0.24% total titrateable acidity following 15 days of storage. Hong *et al.* (2012) described that during storage in guava chitosan coated fruit has lower acidity loss and may take part to delay the fruit senescence. Our findings showed similarities with the study of earlier worker (Nongtaodum *et al.*, 2009) who observed titrateable acidity decreased in coated fruits as compared to control.

Retention of titrateable acidity has been described for different fruits preserved with edible coatings (Ali *et al.*, 2011; Yaman *et al.*, 2012).

Ascorbic acid

Maximum fruit ascorbic acid (231.60 mg/100g) was recorded in chitosan 40 mg (T3) which was statistically at par with T2 (217.27 mg/100g) and T1 (215.00 mg/100g) while minimum (197.80 mg/100g) was recorded in control (T0) after 15 days of storage respectively.

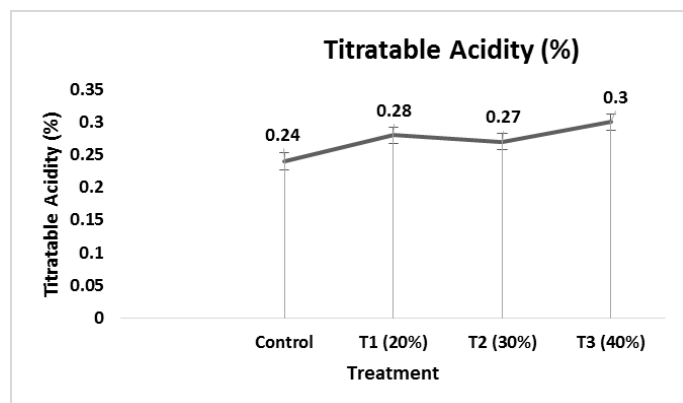


Figure 6: Effect of chitosan coatings on titerable acidity of guava.

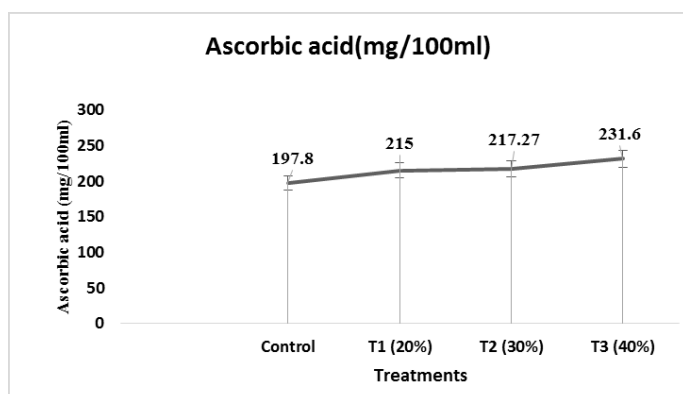


Figure 7: Effect of chitosan coatings on Ascorbic acid of Guava.

Fruit surface pores concealed due to various concentrations of chitosan, so crossing of oxygen is controlled (Zhang *et al.*, 1997). Thus, the oxidizing of ascorbic acid may be effected (Wahab *et al.*, 2012). Results of our study are close with the findings of earlier workers (Trung *et al.*, 2011; Ali *et al.*, 2011). They observed that chitosan treated fruits showed high value of ascorbic acid (Figure 7).

Total sugars

The total sugars of the guava fruits increases till the end of the storage duration except untreated fruits under cold storage condition. The maximum mean total sugars (6.97%) were recorded in (T0) control after 15 days of storage. Minimum total sugar percentage was recorded in (T3) 40% having 6.6 %. Total sugar content increases may be because alteration of starch into sugars. Kaur *et al.* (2017) noted that total

sugars increased (6.43%) with chitosan application (1.5%). But results are in contrast with findings of earlier worker (Trung *et al.*, 2011) who reported that total sugars were decreased in apple (Figure 8).

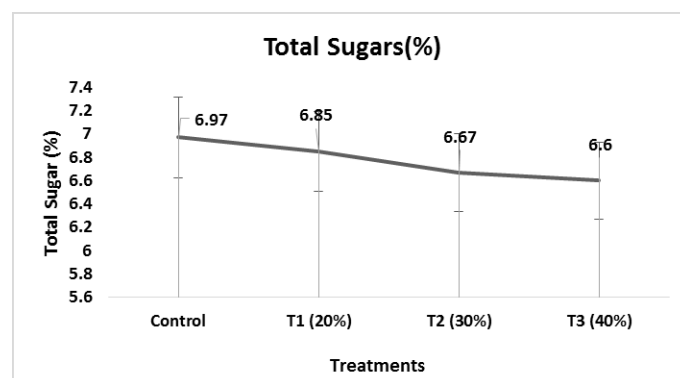


Figure 8: Effect of chitosan coatings on Total sugars (%) of Guava.

Reducing sugars were recorded after 15 days of cold storage (11°C). Figure 9 showed the data about the correlation of average reducing sugar with the four various intensities of chitosan.

Maximum reducing sugars 3.82% was noted in Fruits treated with 40% chitosan (T3) showed maximum reducing sugars 3.82%, followed by 30% (T2) which showed 3.79 % reducing sugar. Minimum reducing sugar percentages were recorded for 20% chitosan (T1) and control (T0) by having 3.57 % and 3.48 % reducing sugars after 15 days of storage respectively. During the storage reducing sugar increases possibly due to the enzymatic alteration of starch to reducing sugar.

Finding of present study were in support of findings of earlier worker (Chailoo *et al.*, 2008) who observed that coated sweet cherries had increased reducing sugar percentage. This view concurs to the findings of Abbasi *et al.* (2009) observed that the postharvest nature of mango fruit effected by chitosan coating and also detected that the total sugar, reducing sugar and non-reducing sugar contents were less in the coated samples (Figure 9).

Non reducing sugars

Figure 10 give the data regarding assessment of non reducing sugars via the applications of four various level of chitosan. Maximum non-reducing sugars 3.49 % was seen in control (T0) after 15 days of storage subsequently, 30% (T2) which presented 3.28 % non-reducing sugars. After 15 days of storage 2.78% minimum non reducing sugar was recorded in T3 (40%).

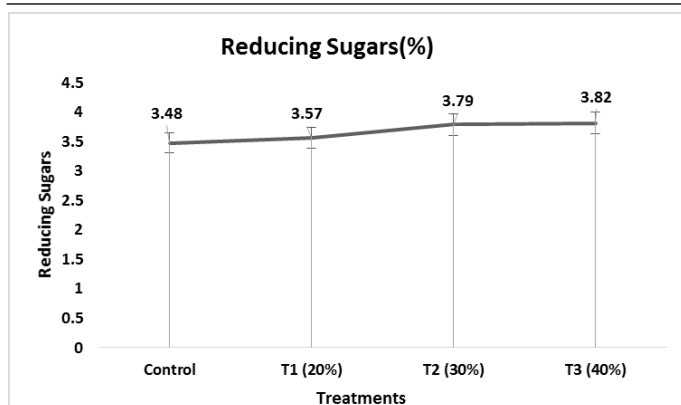


Figure 9: Effect of chitosan coatings on Reducing sugars of Guava. Reducing sugars

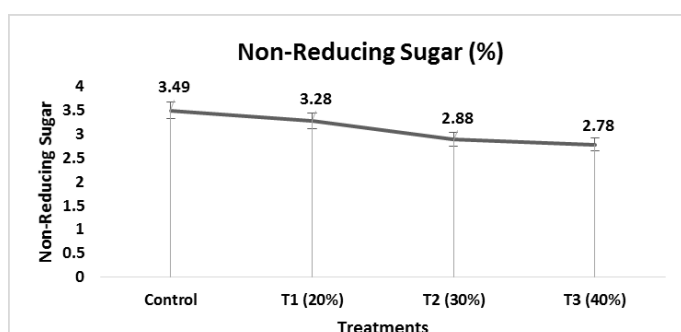


Figure 10: Effect of chitosan coatings on Non Reducing Sugars of Guava.

Our findings were parallel to the consequences of Hoa and Ducamp (2008) on 'cat Hoa loc' mango where they observed the impact of various coatings and perceived that contents of total sugar, reducing sugar and non-reducing sugar were minimum in the coated fruit. Our findings are in close with earlier worker (Ali *et al.*, 2011).

Total phenolic compounds

Phenolic compounds has high biological activity in cancer and heart diseases anticipation (Rocha *et al.*, 2007). Treatments exerted a significant influence on total phenolic compounds (Figure 4 and 11). Highest phenolic compounds was noted in fruits treated with 40% chitosan having 340.67 mg GAE/ml after 15 days of storage and as well also 30% chitosan (T2) which indicated 325.67 mg GAE/ml phenolic compounds. T2 and T3 both indicated similar results. 20% chitosan (T1) and control (T0) vary non-significantly by having 318.67 mg GAE/ml and 300.67 mg GAE/ml phenolic compounds respectively.

Our results are in contrast as compare to findings of earlier workers (Sanchez *et al.*, 2011) in grapes. They found that phenolic compounds increased with chitosan coatings in grapes.

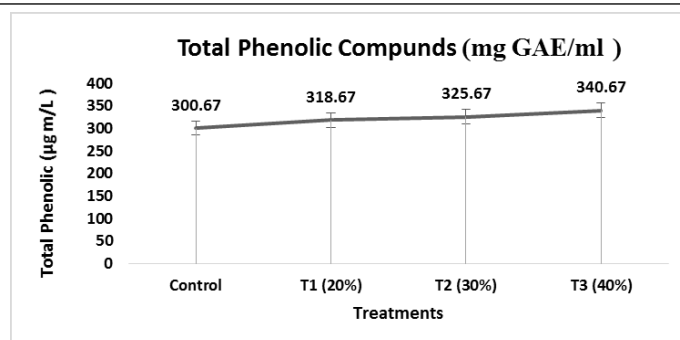


Figure 11: Effect of chitosan coatings on Total Phenolic Compounds of Guava.

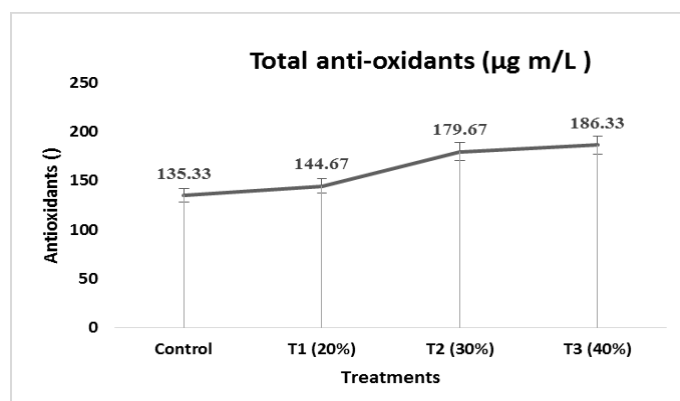


Figure 12: Effect of chitosan coatings on total antioxidants of guava.

Total anti-oxidants

Total anti-oxidants were recorded after 15 days of cold storage (11°C). Figure 12 revealed that maximum anti-oxidants 186.33 µg/ml were in 40% chitosan (T3) treated fruits followed by 30% chitosan (T2) that revealed 179.67 µg/ml anti-oxidants. Control (T0) having anti-oxidants 135.33 µg/ml and vary non-significantly from each other after 15 days of storage. Same findings were found in grapes (Sanchez *et al.*, 2011).

Conclusions and Recommendations

Chitosan coating, particularly 40% chitosan coating combined with a cold storage conditions (11 °C) slow down the fruit maturity and as well as retained the quality of the guava fruit. The results proposed that chitosan demonstrated encouraging effect in keeping membrane integrity and reduction in oxidative stress mostly delayed maturity development in guava fruit.

Acknowledgments

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Novelty Statement

Our research work evidently demonstrated that chitosan can be utilized as a successful additive to enhance the quality and time span of usability of different fruits. Moreover, main purpose of this study was to evaluate prospective impact of chitosan treatments on shelf life of guava cultivar 'Go-la' and quality aspects in cold storage. It has no side effect on human health and a unique product for commercial purpose.

Author's Contribution

Amina: Wrote the manuscript.

Muhammad Zahid Rashid: Performed laboratory work and statistical analysis.

Amina Rashid: Removed the plagiarism in manuscript and performed bio-chemical analysis.

Conflict of interest

The authors have declared no conflict of interest.

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